

ANDREW GOBEA:

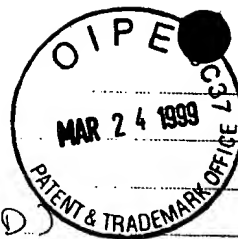


EXHIBIT
1

Received w/c cold blood (w/c PD)

PE mms 30%

PE 70%

added 15 ml 1xPBS to bring to incubation volume of 45 ml.

Added 1.5 ml antibody (12.8)

incubated 25 ml.

Primed cellpro "ceprate".

spin cells. Resuspended in 1x PBS to a final volume of 300 cc in bag.

Ran through column.

Unadsorbed portion → spun down and consolidated in 1x PBS for incubation.

75 ml for incubation (added HPA)

1.5 ml antibody (12.8) 25 min. Spun down

following incubation. Rpt to vol. of 300 cc in bag. Ran through 2nd column.

stem cell portions from Runs 1 & 2 were combined (after counts done and samples removed for staining)

total cells 2.8×10^6 for transduction

BBmm +

IL3 SANDO

WANT

IL6 SANDO

WANT

SLF ANTI

WANT

final concn

will be dil

Cells are

concentrated

BBmm: FB

BS

2.8×10^6 cells

Put in

BBMM + 3161SCF (for 500ml of media)

IL3 SANDOZ # 40230892 stock at 150ug/ml
want final: 20ug/ml x 2 ∴ 20ug

133ul add

IL6 SANDOZ # 40450392 stock at 150ug/ml
want final: 50ug/ml x 2 ∴ 50ug

333ul add

SCF AUBIEN # 1509F2 stock at 1.5mg/ml = 1500ug/ml
want final: 100ug/ml x 2 ∴ 100ug

67ul add

final concentrations are doubled since the media
will be diluted 1:2 w/ viral supernatant.
Cells are therefore incubated with the correct
concentrations.

BBMM: FBS Gemini lot # A8603H
BSA #115

2.8×10^6 cells want final: 5×10^4 cells.

Put in 2 T-75 30ml each: 15ml B365 051193
15ml LPSU^{G7} lot # 53

+ protamine sulfate 240ul
of 1:10 diluted 50

pin down
if 300u in bag.

1 1/2
more
and

Cord Blood cells pre processing:

CFUs:

SET 143

Start:

Plate #	Sample	# Cells	# ul/ml media
- G418	1ab	5×10^4	50
+ G418	2ab	\downarrow	50
- G418	3ab	1×10^5	100
+ G418	4ab	\downarrow	100

adsorbed
fraction:

adsorbed
fraction

CFUs Post transduction: SET 144

plate #	# Cells	# ul
- G418	500	7
\downarrow	1000	14
+ G418	2000	28
\downarrow	500	7
	1000	14
	2000	28

(yields)
adsorbed

count:

$$\bar{x} = 34$$

$$\times 2 \times 10^4 = 6.8 \times 10^5 \text{ Clml}$$

$$\times 5.5 \text{ ml} = 3.7 \times 10^6 \text{ C}$$

Reinforced on 5/15/93
nontransduced 4500 cells

G418

-

+

7ab
8ab

1000
2000

20
40

adsorbed
fraction from
media

Start:

$5 \times 10^8 \text{ c}$

PRE

0.71%

Post ab

0.22%

*ul/ml media

$*34+ = 3.6 \times 10^6 \text{ c}$

$= 1.1 \times 10^6 \text{ c}$

adsorbed
fraction #1:

$2 \times 10^6 \text{ c}$

FL1 FL2 gate

31.94%

FL1 FSC gate

20.81%

$*34+ = 0.64 \times 10^6 \text{ c}$

$= 0.42 \times 10^6 \text{ c}$

adsorbed
fraction #2:

$0.8 \times 10^6 \text{ c}$

2.46%

5.80%

$*34+c = 0.02 \times 10^6 \text{ c}$

$0.05 \times 10^6 \text{ c}$

(yields)

adsorbed #1:

PRE & FL1/FL2 gate

$\frac{0.64 \times 10^6 \text{ c}}{3.6 \times 10^6 \text{ c}}$

$= 17.8\%$

PRE & FL1/FSC gate

$\frac{0.42 \times 10^6 \text{ c}}{3.6 \times 10^6 \text{ c}}$

$= 11.7\%$

post ab & FL1/FL2 gate

$\frac{0.64 \times 10^6 \text{ c}}{1.1 \times 10^6 \text{ c}}$

$= 58.2\%$

Post ab & FL1/FSC gate

$\frac{0.42 \times 10^6 \text{ c}}{1.1 \times 10^6 \text{ c}}$

$= 38.2\%$

adsorbed #2:

PRE & FL1/FL2

PRE & FL1/FSC

~~removed from~~
~~medium~~~~medium~~

ZACHARY RIGGINS:

5/14/93

RECD 200cc COLD BLOOD

PRE: mono poly
 109 109

$$218 \times 50 = 10.9 \times 10^6 \text{ clml}$$

$$\times 200 \text{ ml} = 2.2 \times 10^9 \text{ C} \quad \text{start}$$

Added 3 vials (4.5 ml) 12.8 ab.
 inc. 25 min.

Spindown Rspd in 1x PBS to 300ml
 in bag.

Ran through column:

Spun down unadsorbed fraction for 2nd ab
 incubation.

Spun stem cell fraction to Rspd in
 smaller volume for count.

Counts:

unadsorbed

mono poly
 67 102

$$109 \times 50 \times 10^3$$

$$= 8.5 \times 10^6 \text{ clml} \times 225 \text{ ml}$$

$$= 1.9 \times 10^9 \text{ C}$$

stem

mono poly
 172 16

$$188 \times 2 \times 10^4$$

$$= 3.8 \times 10^6 \text{ clml} \times 5.5 \text{ ml}$$

$$= 20.7 \times 10^6 \text{ C}$$

incl
 12.8
 spu
 Rsp
 Ran

COX

1

2

3

3.

=

per
 freeze

com

26x1
 was
 = 2 =

13.51

LAST

incubated unadsorbed fraction w/ 4.5 ml
12.8 ab. for 25 min -
Spun down.

Put in 300ml in bag (w/ 1x PBS)
Ran through 2nd column.

Counts:

unadsorbed
monos polys
30 33

stem
monos polys
58 4

start

$63 \times 53 \times 10^3$

$62 \times 2 \times 10^4$

$3.15 \times 10^6 \text{ cpm} \times 600 \text{ ml}$

$= 1.2 \times 10^6 \text{ cpm}$

$= 1.9 \times 10^9 \text{ c}$

$\times 5 \text{ ml} = 6 \times 10^6 \text{ c}$

↓

200ml

percolled/ficoll
free \Rightarrow LWT2)

combined stem cell fractions

100 ab

$26 \times 10^6 \text{ c}$ for transduction

want final $\text{EJ} = 5 \times 10^4 \text{ cpm}$

520 ml total

$= 2 = 260 \text{ ml sup}$

260 ml media

13 flasks 40 ml/flask

20 ml sup

20 ml media (B365)

+ 300 ml protamine sulfate

170

up

LASN sup 539 (bottles 18/19)

2×10^4

ml $\times 55 \text{ ml}$

?

CFUs:

5/17/45

PRE

plate #

Sample

cells

ill

lab

2ab

 (-G418)
 PRE + G418
 ↓ (+G418)
 5×10^4

↓

5

5

5/17

CAR

PRE

PRE

PRE

BBMM + 3/16/50F:

1L3 Sandoz # y0230392

1L6 Sandoz # y0450392

SCF AMGEN # 1509152

OG

↓

+G418

↓

OG

+G418

Took sample to micro for sterility ✓
 each day of transduction pt.
 Stat Gram stain done (negative)
 before cells were given to baby.

5/15/93 4pm 2nd transduction:

Spun cells down from each flask
 Respd in fresh media & LASN supe
 added Protamine sulfate

5/16/93 3rd transduction 330pm.
 Repeated above.

517193 cells washed 4x
 3x in 1x PBS + P15
 last wash in RPMI (no p15)

cell

5
5count: 60×10^6 c

$$\bar{x} = 15 \times 10 \times 10^4 = 15 \times 10^6 \text{ c}$$

$$\times 40 \text{ ml} = 60 \times 10^6 \text{ c}$$

Put in 5cc into 10cc syringe

Reinfused on 517193 (UCSF)

Post trans. CFUs: 50746

sample plate #		# cells	# cell
OG418	1ab	500	4
↓	2ab	1000	8
↓	3ab	2000	16
+G418	4ab	500	4
↓	5ab	1000	8
↓	6ab	2000	16
OG418	7ab	1000	24
+G418	8ab	1000	24

ty ✓
 wire)
 o baby
 lion:
 in flock
 on supe

330pm..